

DEPARTMENT OF HEALTH AND HUMAN SERVICES

NOTE TO THE FILE (BNF0018)

Subject: Insect Protected Corn

Keywords:

Corn, Insect resistant, *Bacillus thuringiensis* subspecies *kurstaki*, *cryIA(b)*, Insect control protein cryIA(b), *Ostrinia nubilalis*, European Corn Borer, *hsp70* intron, Glyphosate tolerant, Herbicide tolerant, CP4 EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS), *gox*, glyphosate oxidoreductase, *nptII*, neomycin phosphotransferase II (NPTII)

Background

In submissions dated September 15, 1995, March 14, 1996, and May 24, 1996, Monsanto Company provided summary information to support their safety and nutritional assessment of new corn lines (hybrids) containing transformation event MON801.

Intended Effect and Food/Feed Use

The intended technical effect of this genetic modification of corn is to confer resistance to the feeding of the European Corn Borer, *Ostrinia nubilalis*, a lepidopteran pest insect. The modified corn lines may also resist the feeding of other lepidopteran pest insects, such as the southwestern corn borer, armyworm, corn earworm, fall armyworm, and stalk borer. Monsanto states that insect resistance is conferred by the insecticidal protein cryIA(b), which is reported to be identical to that found in nature and in commercial insecticidal formulations obtained from *Bacillus thuringiensis*. Tolerance to the herbicidal compound glyphosate was utilized as a selectable marker during the development of MON801 corn. The firm indicates that the protein conferring herbicide tolerance, 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS), is identical to that present in Roundup Ready™ soybeans and has been previously reviewed by the Agency.

The firm reports that 50 to 60% of the corn grain produced in the United States is used for animal feed, but the crop also has human food uses. Human food products, which are obtained from corn grain, include starch, high fructose corn syrup, ethanol, and corn oil. Whole plant corn silage is a major ruminant feedstuff.

Molecular Alterations and Characterization

The novel genetic material contained in line MON801 was inserted into the corn genotype, high Type II crossed with FRB73, using particle acceleration transformation. The transformation vectors were plasmids PV-ZMBK07 and PV-ZMGT10. Monsanto reports that the modified lines contain the CP4 EPSPS and *gox* genes, which code for proteins that confer tolerance to the herbicide glyphosate. The plants also contain the insect control gene *cryIA(b)* and *nptII*; the latter codes for neomycin phosphotransferase II (NPTII). NPTII is used as a selectable marker in bacterial systems. The firm states that

nptII is under the control of a bacterial promoter and thus, will not be expressed in corn. Monsanto reports that Southern blot analysis indicates that MON801 expresses the entire *cryIA(b)* protein.

Monsanto reports that there are two insertion sites in MON801 corn, as determined by Southern blot analyses of leaf tissue. Both insertions contain fragments of each plasmid. One insert is made up of a partial copy of *cryIA(b)* and the entire *gox* gene. The second has a complete copy of *cryIA(b)*, two partial copies and one complete copy of *CP4 EPSPS*, one incomplete *gox* gene, and one partial and one complete sequence for *nptII*. Monsanto indicates that, based on nucleotide sequence homology, the incomplete *cryIA(b)* gene present in the first insert would not produce a protein with insecticidal activity. The plant genomic DNA associated with this partial *cryIA(b)* gene insert exhibited no homology with any nucleotide sequence present in the GenBank. The DNA sequence analyses of all breakpoints in the inserts suggested that the incorporation of foreign genetic material into the MON801 corn line did not raise any safety concerns. Monsanto reports that Western blot analyses confirm that only the complete *cryIA(b)* and *CP4 EPSPS* proteins are present in MON801. The firm reports that Mendelian segregation patterns are consistent with their conclusion of a single, active insert for insect resistance as determined by insect feeding trials. The inserted genes are reported to have been stable for at least 5 generations, based on ELISA results.

The *cryIA(b)* gene is reported to be a modified version of the gene present in *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1. The modification was required to improve gene expression in plants. The firm states that the amino acid sequence of the expression product is identical to that of the native protein. Inserted between the *cryIA(b)* gene and its E35S promoter is the intron from the corn *hsp70* gene. The intron was introduced to increase transcription levels of *cryIA(b)*.

The *CP4 EPSPS* gene is utilized as a selectable marker for transformed corn plants. The gene is reported to be completely sequenced and was isolated from *Agrobacterium* strain CP4. To exhibit herbicide tolerance, the *CP4 EPSPS* protein must be translocated into the chloroplast. To achieve this, the chloroplast transit peptide coding sequence (CTP2) from the *Arabidopsis thaliana* *EPSPS* was fused to the N-terminus of the *CP4* protein. The transit peptide is cleaved from the enzyme after arrival in the chloroplast. In addition to *CP4 EPSPS*, MON801 plants also contain the *gox* gene, isolated from *Achromobacter* strain LBAA, as another selectable, herbicide tolerance marker. The gene codes for glyphosate oxidoreductase, an enzyme that degrades glyphosate. The *gox* enzyme also requires translocation into the chloroplast for activity. Translocation is accomplished by use of another transit peptide, CTP1, derived from the ribulose-1,5-bisphosphate carboxylase (*SSU1A*) gene from *Arabidopsis thaliana*. The *hsp70* intron was also used to increase transcription levels of this herbicide tolerance gene. However, Monsanto reports that although *CTP1* and *gox* genes are present in MON801 corn, the enzyme is not expressed, as determined by Western blot analyses of leaf tissue and grain (limit of sensitivity - 1 ng/ 30 µg total protein). The lack of *gox* expression was confirmed by ELISA analysis of leaf, grain, and whole plant extracts. The firm also reports that Western blot analysis failed to detect the *NPTII* protein.

Expressed Proteins

Monsanto reports that both expression products (cryIA(b) and CP4 EPSPS) are only a small portion of plant protein. CryIA(b) makes up 1.3, 0.57, and 1.77 $\mu\text{g/g}$ fresh tissue, while CP4 EPSPS represents 1.85, 4.11, and 0.57 $\mu\text{g/g}$ of leaf, seed, and whole plant tissue, respectively. The firm reports that cryIA(b) is not present in MON801 pollen. The firm does not address the presence of CP4 EPSPS and cryIA(b) in corn oil which would be unlikely since the proteins should be eliminated during oil processing. As discussed previously, Monsanto indicates that the *gox* and *nptII* genes are not expressed in MON801 corn.

Regulatory Considerations

Monsanto states that the cryIA(b) protein present in MON801 is identical to that present in nature and commercial microbial preparations approved by the EPA. The safe use of insecticidal proteins as pesticides and the use of selectable markers as pesticidal inert ingredients in the development of insect resistant plant varieties are regulated by the Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). Therefore, we have not addressed the safe use of cryIA(b) as a pesticide or the safe use of CP4 EPSPS as a pesticidal inert ingredient.

Nutritional Assessment

Grain

Based on the nature of the genetic modification, it was expected that MON801 corn would not materially differ in composition from other corn varieties. To confirm this expectation, Monsanto analyzed the nutrient composition of grain obtained from MON801 corn and a comparable control, MON800, by standard methods for protein, fat, ash, carbohydrate, moisture, calories, amino acid profile, and fatty acid composition. Grain samples were also analyzed for carbohydrate composition and amounts of phytic acid, tocopherol, calcium, phosphorus, and aflatoxins.

No statistically significant differences are reported by Monsanto for the following grain analytes: fat, ash, moisture, calories, most amino acids, fatty acids, carbohydrate composition, alpha and beta tocopherol, phytic acid, calcium, phosphorus, and aflatoxins. The firm indicates that statistical differences did exist between modified and control line for protein, carbohydrate, tryptophan, isoleucine, valine, leucine, and glycine, but that all values fell within the ranges reported in the literature for corn. Levels of gamma tocopherol were significantly lower in the modified corn line, but Monsanto states that this isomer is one tenth as active as the alpha form and thus, is not an important constituent of corn grain.

Vegetative Tissues

Compositional analyses of green chop (silage) included protein, fat, ash, carbohydrate, neutral detergent fiber, acid detergent fiber, and dry matter in four field trials. In another experiment, analytes included calories, crude fiber, calcium, and phosphorus. Monsanto indicates that forage produced by MON801 corn is similar to that of the control lines.

Wholesomeness Experiments

Monsanto also conducted several wholesomeness trials using MON801 grain. Rats were fed a rodent diet containing 32.8% corn meal for 4 weeks. Analytes included: intake, gain, mortality, "clinical signs", and absolute and relative organ weights. The firm reports that there were no significant differences between the treated and control groups, although MON801 female animals consumed significantly less feed during the first 3 weeks. The firm states that there was no effect on growth or nutritional status, despite the lower level of intake in these rats.

A second trial was conducted using fingerling catfish consuming isonitrogenous diets containing 35% ground corn. Monsanto reports that feed per fish, conversion ratios, final weight, percentage gain, and survival did not differ between fish receiving MON801 or control corn. Tissue analytes included protein, fat, moisture, and ash. Fish fed MON801 corn did exhibit a higher muscle protein content (dry matter basis), but the firm concluded that the small difference was not meaningful. Other tissue analytes and protein content, expressed on a wet basis, did not differ between the two groups.

Bobwhite quail were fed diets containing 5 and 10% raw corn meal from MON801 and MON800. The firm reports that none of the birds died during the trial. Appearance and behavior are reported to have been normal and there were no differences in intake or body weight.

Monsanto concludes this section by stating that the absence of unintended effects in MON801 corn grain is demonstrated by: 1) the safety of the host, corn; 2) compositional analyses; and 3) the wholesomeness trials.

Conclusions

Monsanto has concluded that "commercialization of Line 801 is fully consistent with the FDA Food Policy and with the requirements of the Federal Food, Drug, and Cosmetic Act." At this time, based on Monsanto's description of its data and analyses, the Agency considers Monsanto's consultation on corn lines containing transformation event MON801 to be complete.

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